

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

MICHAEL BLABER ET AL.

Serial No. 10/037,633

Filing Date: January 3, 2002

**For: SYNTHETIC NUCLEIC ACID
SEQUENCES FOR 2,5-DIKETO-D-
GLUCONIC ACID REDUCTASES
AND ASSOCIATED METHODS**

Examiner: Richard D. Hutson Ph.D.

Art Unit: 1645

DECLARATION UNDER 37 CFR 1.132

Sir:

I hereby declare that:

1. I am the named inventor in the above referenced patent application.

2. The claimed invention is non-obvious over the cited references to Anderson et al. (*Science*, Vol. 230, pp. 144-149, 1985), in view of Mohsen et al. (*Gene*, Vol. 160, pp. 263-267, 1995), and as evidence thereof I respectfully submit the following observations regarding the cited references.

3. The Examiner has taken the position that Anderson *et al.* teach the isolation and cloning of the cDNA for DKGRA. This view is not quite correct. The isolation of the wild type DKGR gene was from the natural *Corynebacterium* host, where replication is *de facto* not a problem. The gene was identified by hybridization techniques that do not require polymerase-based manipulations. Anderson *et al.* report that sequencing, a polymerase-based manipulation, was "difficult." Anderson *et al.* also report subcloning the DKGR gene into an *E. coli* expression vector, again a procedure that does not require

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polymerase-based manipulations, with subsequent expression attempts in the *E. coli* host having failed even with a strong expression promoter in the expression plasmid. Anderson *et al.* postulated that the lack of expression may be due to a dyad symmetry, a palindrome, at the 5' Shine-Dalgarno sequence. This statement shows that Anderson *et al.* believe that the poor expression of the wild-type DKGR gene in *E. coli* is due to secondary structure in the mRNA caused by a palindromic sequence, and not due to the G+C content of the gene (see p.147 of the reprint). This palindromic sequence region is outside of the coding sequence, in fact it is in the 5' untranslated region and is not part of our synthetic gene sequence. Accordingly, Anderson *et al.* do not address the problem on which the present invention is focused, nor do they describe any teaching that would lead the skilled to the present invention.

4. The patent examiner also expresses the view that the Mohsen *et al.* refereece teaches that high-level expression of the human isovaleryl-CoA dehydrogenase (IVD) gene in *E. coli* can be achieved by altered codon usage of a region with high G+C content to mimic codon usage of highly expressed proteins in *E. coli*. This view of the Mohsen *et al.* reference is not correct, and the reference is inapplicable to the present invention for the following reasons.

First, the report of Mohsen *et al.* does not detail problems associated with polymerase-based manipulations (e.g. PCR, or nucleic acid sequencing) that are due to high G+C content - only expression-based issues. One of the main problems with the *Corynebacterium* DKGR gene was an inability to engage in polymerase-based manipulations that are essential for in vitro genetic engineering. We hypothesized that this was due to high G+C content throughout the gene. We demonstrated that reduction of

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G+C content allowed efficient polymerase-based manipulations. This point is not described or even suggested in the Mohsen *et al.* reference.

Second, the IVD gene studied by Mohsen *et al.* does not contain high G+C content throughout, but only in the 5' region is there high G+C content. Mohsen identifies this region as a mitochondrial targeting domain (comprising only 87 base pairs, or 29 amino acids) on the 5' end of the gene. The work of Mohsen *et al.* thus teaches that human 5' mitochondrial targeting domains (which are problematic for expression in *E. coli*) can be rendered non-problematic by reduction of G+C content. Since *Corynebacteria* do not contain mitochondria, the problem with DKGR expression in *E. coli* does not involve a 5' mitochondrial targeting domain, and Mohsen's teaching is irrelevant to the problem solved by the present invention. In *Corynebacteria*, the G+C content is distributed throughout the DKGR gene and is, therefore, a systemic genetic issue and not one of a short 5' eukaryotic functional domain that complicates expression in prokaryotes. Furthermore, as noted above, the problem with the systemic G+C content of DKGR is the difficulty in carrying out polymerase-based manipulations with the wild-type gene.

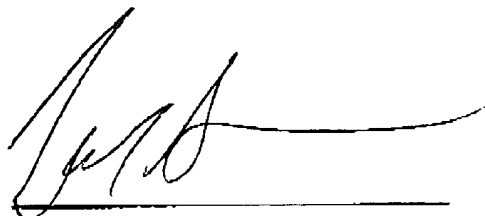
Third, since the 5' mitochondrial targeting domain of the IVD is reported by Mohsen to be the problem for expression, those skilled in the art would reasonably assume that cleavage or removal of this domain would render the remaining portion of the enzyme non-problematic with regard to expression in *E. coli*. Because the mitochondrial targeting domain in IVD does not appear to be part of the essential enzymatic portion of the IVD protein, elimination of the domain may have sufficed to obtain expression. This is what Mohsen teaches, and it is not related to the present application, nor would it suggest the present invention to the skilled.

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I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9/4/03

Date



Michael Blaber